AMERICAN JOURNAL OF PHARMTECH RESEARCH
Journal home page: http://www.ajptr.com/

Analytical Method Development and Forced Degradation Studies on Proteasome Inhibitor: Bortezomib As A Part of Preformulation Study to Develop Stable Lyophilized Dosage Form

Yaswanth Allamneni[*1], T.E.G.K Murthy[2], Mandava Venkata Basaveswara Rao[3], Y Udaya Bhaskara Rao[4]

1.Department of Pharmacy, Krishna University, Machilipatnam, Andhra Pradesh, India.
2.Bapatla College of Pharmacy, Bapatla, Andhra Pradesh, India.
3. Department of Chemistry, Krishna University, Andhra Pradesh, India.
4. SP Accure Labs Private Limited, Thurakapally, Hyderabad, Telangana, India.

ABSTRACT

Forced degradation study is an important tool to identify the degradation of the drug substance[1] when subjected to different stress conditions which is more useful than the accelerated stability testing during the formulation development. A simple and stability indicating HPLC method was developed for the characterization of assay and related substances of the drug substance. In the present investigation, bortezomib was subjected to different stress conditions like acid hydrolysis, base hydrolysis, oxidation, effect of heat and effect of light. Significant degradation was observed when Bortezomib sample solutions are exposed to acidic conditions and basic at room temperature. Drastic degradation was observed when bortezomib sample solutions are exposed to oxidation. Significant degradation was observed when bortezomib sample solution is exposed to heat at 60°C and exposed UV radiation. Solid state degradation data indicates that bortezomib has insignificant degradation upon exposure to UV light. The proposed analytical methods enable accurate, precise, and rapid analysis of Bortezomib. Stress study results helps in opting the solvents for the compounding process with respect to stability of the compound. Based on the stress study data, the product can be lyophilized in clear glass vial for the future prospective of novel lyophilized formulation.

Keywords: forced degradation study, preformulation study, proteasome inhibitor and stress conditions.

*Corresponding Author Email: yaswanthallamneni@gmail.com
Received 17 April 2017, Accepted 22 April 2017

Please cite this article as: Allamneni Y *et al.*, Analytical Method Development and Forced Degradation Studies on Proteasome Inhibitor: Bortezomib As A Part of Preformulation Study to Develop Stable Lyophilized Dosage Form. American Journal of PharmTech Research 2017.

INTRODUCTION

The development of efficacious dosage form depends upon the physico-chemical properties of the drug substance. In simple words, preformulation study strategy identifies the significant barriers in the product development process. The outcome results of preformulation study helps in selection of suitable stable solvent system, compounding procedure, filtration, filling and freeze drying steps while developing the stable lyophilized dosage form. There are many regulatory guidelines on the forced degradation study which are in generalized approach but they have not provided any practical approach for conducting those studies.

Bortezomib is chemically known as [(1R)-3-methyl-1-({(2S)-3-phenyl-2-[(pyrazin-2-ylcarbonyl)amino]propanoyl}amino)butyl]boronic acid which belongs to the category of modified dipeptidyl boronic acid[3,4]. Bortezomib is a first class reversible inhibitor of 26S proteasome which is indicated for the treatment of patients with multiple myeloma and mantle cell lymphoma. Inhibition of 26S proteasome prevents the targeted proteolysis thereby effects the multiple signaling cascades within the cell. The molecular formula for Bortezomib is $C_{19}H_{25}BN_4O_4$ and its molecular weight is 384.24. The chemical structure of Bortezomib was provided in Figure 1.

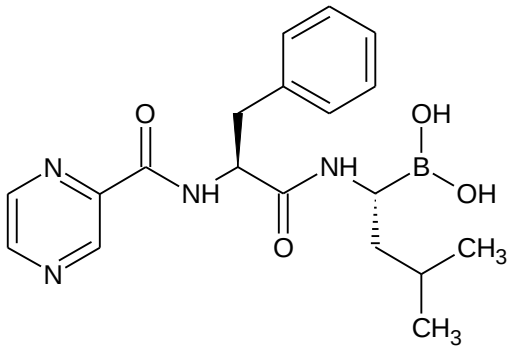

Figure 1: Chemical Structure of Bortezomib

Based on the nomenclature of the drug substance, two chiral centers are present in the molecule i.e one at ‘phenyl alanine moiety’ and another one at ‘amino boronic acid moiety’. Different types of Enantiomers of Bortezomib available are S,R Enantiomer, S,S Enantiomer, R,R Enantiomer and R,S Enantiomer.

MATERIALS AND METHOD

Chemicals and Reagents

Formic acid, Hydrochloric acid and Sodium hydroxide were procured from Merck Specialties Private Limited. Acetonitrile Was procured from J.T Baker. Bortezomib and related samples were procured from Shilpa Medicare Limited.

Instruments and Equipments Details

HPLC with PDA detector (Make: Waters, Model No.: 2998 PDA 2695 pump), Electronic Balance (Make: Mettler-Toledo, Model No.: XS-205 dual range), Laboratory precision oven (Make: Tempo, Model No.: TI-128C S/G), pH meter (Make: Mettler-Toledo, Model No.: FEP20 FIVE Easy Plus PH), Flexible Poly Urethane Isolator (Make: PFI systems Limited, Model No.: IB821), Powered air purifying respirator (Make: BLS Italy, Model No.: JS 3025 PAPR SGE2600 CE), Vacuum oven (Make: Osworld India, Model No.: OVOR-G-11), Infrared spectrophotometer (Make: Perkin Elmer, Model No.: Paragon 1000) and Develosil C8-UG-5 Column (Make: Nomura Chemicals).

Experimental Procedure

Before initiation of the forced degradation studies on dug substance, it is important to check the purity of the received drug substance with respect to some preliminary evaluation tests as a part of pre-formulation study[5]. Hence some preliminary tests like Description, Identification, Solubility, Loss on Drying, Assay and Related substances were tested.

Description

The sufficient quantity of drug substance was spreaded on petridish in flexible isolator and visually observed for its appearance.

Identification

About 3.0 mg of the drug substance was grinded along with about 300 mg of finely powdered and dried potassium bromide in a mortar and pestle. Using hydraulic press and KBr dye set the mixture was compressed into pellet under the pressure of about 8 to 10 tons. 5 scans were made between the 4000 cm^{-1} to 400 cm^{-1} for background and sample.

Solubility

Solubility of Bortezomib was measured in aqueous buffers pH ranging from 1.0 to 8.0 as well as organic solvents at room temperature.

Loss on Drying

Loss on drying test was performed at room temperature over phosphorous pentoxide under vacuum for 3 hrs. Empty weighing bottle was dried for 30 min at room temperature and weight of empty weighing bottle along with its lid (W1) was recorded. About 0.5 g of sample was transferred into a main bottle and the weight (W2) was recorded. By gentle sidewise shaking sample was distributed as evenly as practicable. The loaded weighing bottle was placed in a vacuum oven maintained at room temperature. Place the dry over phosphorous pentoxide (sufficient quantity (approximately 30 to 40 g) in suitable petridish). Vacuum oven was closed and a vacuum of 20 mmHg was applied and allowed to dry for 3

hours. Weight of weighing bottle was recorded along with the dried sample (W3). Drying was continued with frequency of 30 minutes until 2 successive weighing doesn't differ by more than 0.5 mg (W4).

Calculate the loss on drying as below,

Loss on drying (%w/w) = (W4 -W2)* 100 / (W2- W1)

Assay

Mixture of acetonitrile, water and formic acid in the ratio of 80:20:0.1 (v/v/v) respectively was utilized as diluent. Mixture of acetonitrile, water and formic acid in the ratio of 30:70:0.1 (v/v/v/v) respectively was utilized as mobile phase.

Standard Preparation:

About 100 mg of Bortezomib standard was weighed and transferred into a 100 mL volumetric flask and the volume was made up to 100 mL with diluent. 10 mL of the solution was transferred into 100 mL of volumetric flask and the volume was made up to 100 mL with diluent.

Sample preparation:

About 100 mg of Bortezomib sample was weighed and transferred into a 100 mL volumetric flask and the volume was made up to 100 mL with diluent. 10 mL of the solution was transferred into 100 mL of volumetric flask and the volume was made up to 100 mL with diluent.

Chromatographic Conditions:

Column: Develosil C8-UG-5 (150 X 4.6 mm) or its equivalent, Detector Wavelength: 270 mm, Flow rate: 1.0 mL/min, Injection volume: 20 µL, Run time: 20 min, Column temp.: 35°C, Sample temp.: 5°C.

Related Substances

Mobile phase-A:

Mixture of Acetonitrile, water and formic acid in the ratio of 30:70:0.1(v/v/v/) respectively. Filter through 0.45µm membrane filter paper and edges.

Mobile phase-B:

Mixture of Acetonitrile, water and formic acid in the ratio of 80:20:0.1(v/v/v/) respectively. Filter through 0.45µm membrane filter paper and edges.

Table 1: Gradient elution programming for related substances

Time (min)	Mobile phase- A(%v/v)	Mobile phase-B(%v/v)
0	100	0
15	100	0
30	0	100
45	0	100

47	100	0
55	100	0

Standard stock solution preparation:

About 10 mg of Bortezomib was accurately weighed and transferred into a 100 mL volumetric flask containing 5.0 mL of mobile phase-B and further diluted with mobile phase-A.

Reference solution (a):

0.5 mL of standard stock solution was transferred into a 50 mL volumetric flask and dilute with mobile phase-B followed by makeup volume with mobile phase –A.

Impurity –A standard stock solution (freshly prepared):

About 10 mg of impurity –A was weighed accurately and transferred into a 100 ml volumetric flask containing 5 ml of mobile phase –B and make up to volume with mobile phase-A.

Reference solution (b) (freshly prepared):

Transfer 0.5 ml of impurity-A standard stock solution into 50 ml volumetric flask and makeup to volume with mobile phase-A.

Test solution:

Weigh and transfer about 50 mg of sample into a 50 ml volumetric flask. Dissolved in 5 ml of mobile phase-B and dilute to volume with mobile phase-A.

Calculation of Related substances:

%impurity = (AT/AS) × (DS/DT) × (P/RRF)

Where,

AT: Area response of impurity in test solution

AS: Average area response of reference solution (a)

DS: Concentration of reference solution (a) in mg/mL

DT: Concentration of test solution in mg/mL

P: Assay/ potency of Bortezomib standard on as is basis

RRF: Relative response factor

Forced Degradation Studies (or) Stress Testing[6,7]

Forced degradation studies on the drug substance were performed in both solid state form and solution form.

Effect of Acid

10.0 mL of stock solution was transferred into 20 mL volumetric flask and about 0.4 mL of 1.0N hydrochloric acid solution was added. The solution was kept at room temperature and at 60°C separately for initial, one hour, three hours and six hours then neutralized with 0.4 mL of 1.0N

sodium hydroxide solution and diluted to 20 mL with diluent. The resultant solution was subjected for the peak purity and related substances analysis with respect to RRT.

Effect of Base

10.0 mL of stock solution was transferred into 20 mL volumetric flask, added about 0.4 mL of 1.0N sodium hydroxide solution. The solution was kept at room temperature and at 60°C separately for initial, one hour, three hours and six hours then neutralized with 0.4 mL of 1.0N hydrochloric acid solution and diluted to 20 mL with diluent. The resultant solution was subjected for the peak purity and related substances analysis with respect to RRT.

Oxidation Effect

10.0 mL of stock solution was transferred into 20 mL volumetric flask, added about 0.4 mL of 5% hydrogen peroxide solution. The solution was kept at room temperature and at 60°C separately for initial one hour, three hours and six hours and then diluted to 20 mL with diluent. The resultant solution was subjected for the peak purity and related substances analysis with respect to RRT.

Heat Effect

5.0 mL of stock solution was transferred into 20 mL volumetric flask. The solution was kept at 60°C for six hours and then diluted to 20 mL with diluent. The resultant solution was subjected for the peak purity and related substances analysis with respect to RRT.

UV Effect

UV degradation of Bortezomib was examined by exposing the sample solution to UV light for 24 hours. The resultant solution was subjected for the peak purity and related substances analysis with respect to RRT.

Control Sample

Control sample was prepared for this study by diluting 10.0 mL of stock solution into 20 mL in volumetric flask with diluent. All the above solutions were analyzed along with this control sample using a photodiode array detector.

Solid State Stability

The solid state stability of Bortezomib sample was performed by exposing to UV light and Heat.

About 500 mg of the sample was taken in a petridish and exposed to the UV light for 24hrs. After exposure, about 100 mg of the sample was weighed and transferred into a 100 mL volumetric flask, dissolved and diluted to volume with diluent.

About 500 mg of the sample was taken in a petridish and exposed to heat at 80°C for 24hrs. After exposure, about 100 mg of the sample was weighed and transferred into a 100 mL volumetric flask, dissolved and diluted to volume with diluent.

RESULTS AND DISCUSSION

Description

The appearance of the drug substance was found to be white colored powder.

Identification

FT-IR spectrum of Bortezomib reveals that the characteristic bonds present in the structure were exhibited at their frequency. The results of IR spectrum were depicted in Figure 1 & Table 2.

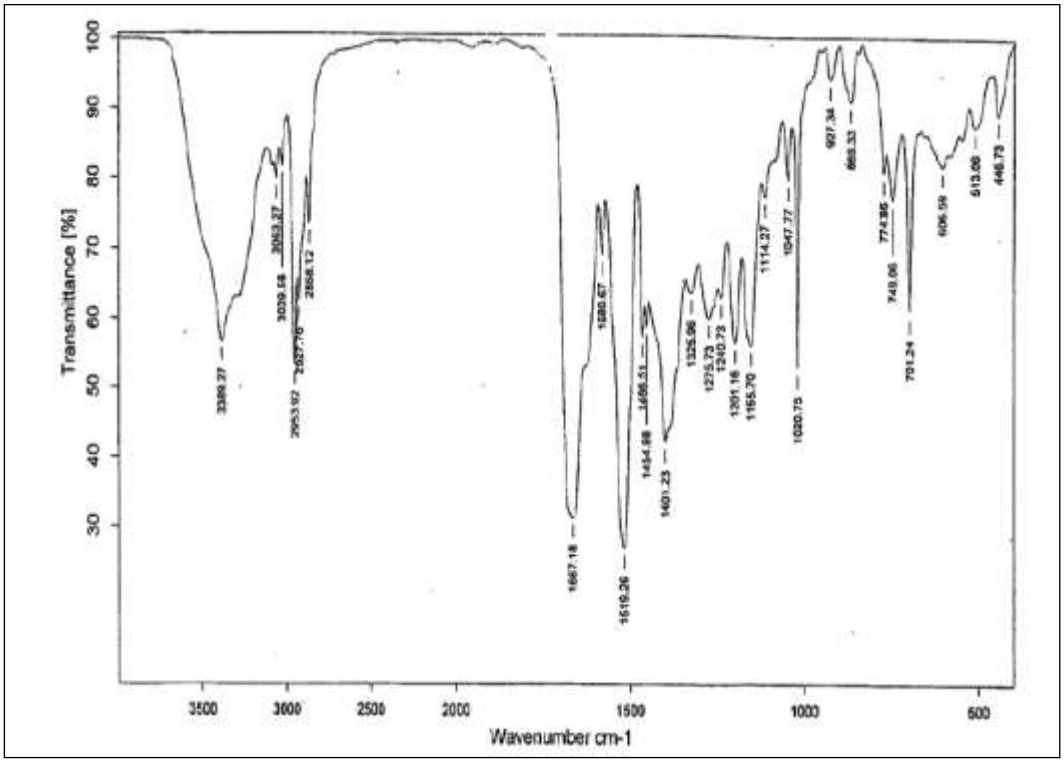

Figure 2: FT-IR Spectrum of Bortezomib

Table 2: Interpretation of IR Spectrum Results

S. No.	Frequency (cm^{-1})	Assignment
1.	3389.28	Amides NH and-OH
2.	3063.26	Aromatic C-H
3.	2953.90	Aliphatic C-H
4.	1667.20	Amides – CO –
5.	1580.68	Aromatic C = C
6.	774.98 and 749.04	Mono substituted Benzene

Solubility

The drug substance exhibits poor aqueous solubility and poor solubility across the physiological media pH range. The drug substance was found to be more soluble in Dimethyl sulfoxide, Dimethyl Formamide and Tetrahydrofuran. The solubility profile of the drug substance was evaluated in different solvents and the results were depicted in Table 3 and Table 4.

Table 3: Solubility Profile of Bortezomib in different aqueous buffers

S. No.	pH of the Buffer	Solubility
1.	1.0	Insoluble
2.	2.0	Insoluble
3.	3.0	Insoluble
4.	4.0	Insoluble
5.	5.0	Insoluble
6.	6.0	Insoluble
7.	7.0	Insoluble
8.	8.0	Insoluble

Table 4: Solubility Profile of Bortezomib in different Solvents

S. No.	Name of the Solvent	Solubility
1.	Acetone	Slightly soluble
2.	Acetonitrile	Sparingly soluble
3.	Chloroform	Sparingly soluble
4.	Dichloromethane	Slightly soluble
5.	Dimethyl sulfoxide	Freely soluble
6.	Dimethyl Formamide	Freely soluble
7.	Ethanol	Slightly soluble
8.	Ethyl acetate	Sparingly soluble
9.	Hexane	Insoluble
10.	Isopropyl alcohol	Slightly soluble
11.	Methanol	Soluble
12.	Tetrahydrofuran	Freely soluble
13.	Toluene	Insoluble
14.	Tertiary Butyl Alcohol	Soluble
15.	Water	Insoluble

Loss on drying

The loss on drying value of Bortezomib was found to be 2.75%w/w.

Assay

Assay of the received drug substance was found to be 99.8%w/w on dried basis.

Related substances

Impurity-A, Impurity-B and Impurity-C were not detected in the chromatogram. Any other individual impurity (or) single maximum impurity was detected in the chromatogram and its value was found to be 0.02%w/w. Total impurities in the drug substance were found to be 0.05%w/w.

Forced Degradation Study Results

Effect of Acid

Results of the forced degradation study from Acid effect exposed at room temperature (RT) and at 60°C are summarized in Table 5.

Table 5: Effect of Acid on Related substances and Peak purity

Impurity	Control	Initial	At Room temperature			At a temperature of 60°C		
			1 hr	3 hrs	6 hrs	1 hr	3 hrs	6 hrs
Impurity-A	ND	ND	ND	ND	ND	ND	ND	ND
Impurity-C	ND	0.04	0.07	0.09	0.09	0.25	0.79	1.38
Impurity-B	ND	0.72	1.22	1.68	1.76	0.76	0.97	2.10
Unknown-I	ND	0.04	0.08	0.09	0.10	0.21	0.75	1.27
Unknown-II	ND	0.03	0.02	0.04	0.04	0.09	0.27	0.45
% Purity	99.95	99.15	98.41	97.94	97.85	98.48	96.92	94.51
Purity angle	0.857	0.752	0.754	0.752	0.665	0.751	0.682	0.733
Purity threshold	1.250	1.002	1.080	1.220	1.061	1.157	1.145	1.081

ND: Not detected

Effect of Base

Results of the forced degradation study from Base effect exposed at room temperature (RT) and at 60°C are summarized in Table 6.

Table 6: Effect of Base on Related substances and Peak purity

Impurity	Control	Initial	At Room temperature			At a temperature of 60°C		
			1 hr	3 hrs	6 hrs	1 hr	3 hrs	6 hrs
Impurity-A	ND	ND	ND	ND	ND	ND	ND	ND
Impurity-C	ND	0.04	1.75	2.60	3.18	0.68	4.22	8.12
Impurity-B	ND	0.72	0.15	0.28	0.38	12.51	39.18	42.55
Unknown-I	ND	0.04	0.15	0.29	0.46	10.15	ND	ND
Unknown-II	ND	0.03	0.06	0.14	0.22	5.08	25.34	30.92
Unknown-III	0.02	0.05	0.15	0.30	0.35	21.8	17.52	10.75
Unknown-IV	ND	0.03	0.48	0.92	1.18	0.03	0.08	1.09
Unknown-V	ND	ND	0.22	0.45	0.59	0.14	0.26	0.26
% Purity	99.95	99.15	97.05	94.90	95.30	49.42	13.15	7.01
Purity angle	0.857	0.752	0.720	0.685	0.720	0.378	0.072	0.078
Purity threshold	1.250	1.002	1.155	1.035	0.979	0.510	0.287	0.302

ND: Not detected

Effect of Oxidation

Results of the forced degradation study from Oxidation effect exposed at room temperature (RT) and at 60°C are summarized in Table 7.

Table 7: Effect of Oxidation on Related substances and Peak purity

Impurity	Control	Initial
Impurity-A	Not detected	Not detected
Impurity-C	Not detected	0.03
Impurity-B	Not detected	Not detected
Unknown-I	Not detected	Not detected
Unknown-II	0.01	99.82
% Purity	99.95	0.00
Purity angle	0.856	Not applicable
Purity threshold	1.252	

Effect of Heat

Results of forced degradation study from Heat effect exposed at 60°C are summarized in Table 8.

Table 8: Effect of Heat on Related substances and Peak purity

Impurity	Control	Initial
Impurity-A	Not detected	Not detected
Impurity-C	Not detected	3.95
Impurity-B	Not detected	1.92
Unknown-I	Not detected	1.47
Unknown-II	0.01	1.55
Unknown-III	0.01	0.26
Unknown-IV	0.02	1.15
% Purity	99.97	89.8
Purity angle	0.855	0.721
Purity threshold	1.253	1.024

Effect of UV Light

Results of forced degradation study from UV light effect exposed for 24 hours are summarized in Table 9.

Table 9: Effect of UV Light on Related substances and Peak purity

Impurity	Control	Initial
Impurity-A	Not detected	Not detected
Impurity-C	Not detected	0.55
Impurity-B	Not detected	0.06
Unknown-I	Not detected	0.75
Unknown-II	0.01	4.92
Unknown-III	0.01	0.80
Unknown-IV	Not detected	0.44
% Purity	99.95	91.43
Purity angle	0.854	0.715
Purity threshold	1.251	1.003

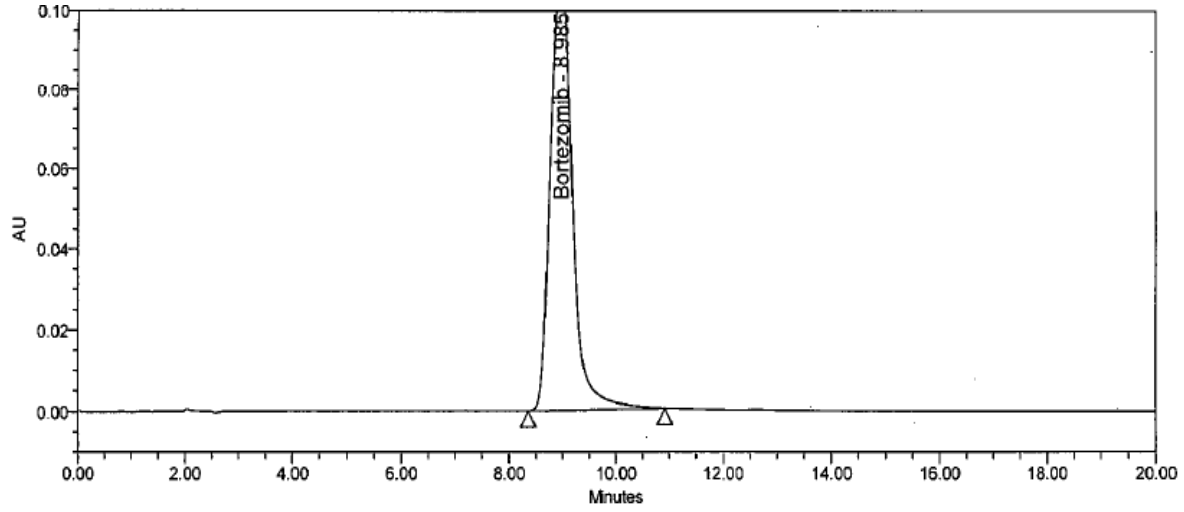

Figure 3: Typical Chromatogram of Bortezomib Assay method

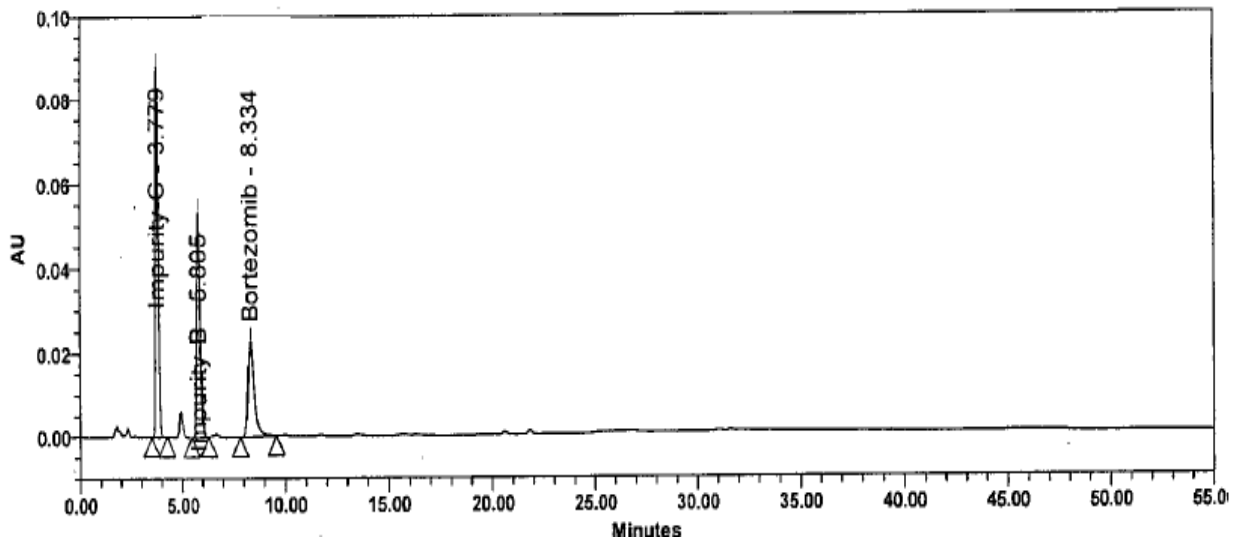

Figure 4: Typical Chromatogram of Bortezomib Related Substances method

CONCLUSION

Effect of Acid: Significant degradation is observed when Bortezomib sample solutions are exposed to acidic condition both at room temperature and at 60°C study by the formation of known impurities Impurity-B and Impurity-C at the level of about 1.38% and 2.10%. One unknown impurity is formed at RRT 0.65 is about 1.27%.

Effect of Base: Significant degradation is observed when Bortezomib sample solutions are exposed to basic conditions at room temperature by the formation of Impurity-C at the level of 3.18% and one unknown impurity at RRT 1.50 is about 1.18%.

Drastic degradation is observed at 60°C study by the formation of Impurity-B, Impurity-C at the levels of about 42.55% and 8.12% and two major unknown impurities at RRT 0.68 is about 30.92% and at RRT 1.25 is about 10.75%. Hence Bortezomib is showing drastic degradation with basic medium at 60°C study.

Oxidation effect: Total Bortezomib molecule is destroyed and formed one major unknown impurity at RRT 1.42 is about 99.82%. So the study is discarded.

Effect of Heat: Significant degradation is observed when Bortezomib sample solutions are exposed to heat at 60°C study by the formation of Impurity-B, Impurity-C are about 3.95%, 1.92% and three unknown impurities are about 1.47%, 1.55% and 1.15%.

Effect of UV Light: Significant degradation is observed when Bortezomib sample solutions are exposed to UV radiation by the formation of Impurity-C about 0.55% and four major unknown impurities are about 0.75%, 4.92,% 0.80% and 0.44% respectively.

This forced degradation study will helps in selecting the solvent system for compounding, processing conditions and temperature during compounding during the development of novel stable lyophilized dosage form for bortezomib.

ACKNOWLEDGEMENTS

Authors wish to give thanks to Management of SP Pharma Private Limited, Hyderabad, India for constant support and given research laboratory to carry out this project work. We also acknowledge the help provided by our colleagues in completion of the project.

REFERENCES

1. Blessy M, Ruchi D Patel, Prajesh N Prajapati, Y K Agrawal. Development of forced degradation and stability indicating studies of drugs - A review. Journal of Pharmaceutical Analysis 2014; 4(3): 159–165
2. M S Charde, Jitendra Kumar, A S Welankiwar, R D Chakole. Review: Development of forced degradation studies of drugs. International Journal of Advances in Pharmaceutics 2013; 2 (3).
3. M N Brinda, V Krishna Reddy, E SasiKiran Goud, Kadarla Vinay Kumar. Development and validation of RP-HPLC method for determination of related substances of bortezomib in injection. World Journal of Pharmacy and Pharmaceutical Sciences 2014; 3(2): 25121-2529.
4. Kasa Srinivasulu, Mopidevi Narasimha Naidu, Kadaboina Rajasekhar, Murki Veerender, Mulukutla Venkata Suryanarayana. Development and Validation of a Stability Indicating LC Method for the Assay and Related Substances Determination of a Proteasome Inhibitor Bortezomib. Chromatography Research International 2012; 1-12.
5. International Conference on Harmonization, ICH Harmonized Tripartite Guidelines-Validation of analytical procedures: methodology, Fed. Regist, 1997.
6. Validation of analytical procedure; text and methodology Q2 (R1), International conference on harmonization of technical requirements for registration of pharmaceuticals for human use. ICH harmonized tripartite guidelines.2005
7. ICH Guidelines on Validation of Analytical procedure: Text and Methodology Q 2 (R1). IFPMA, Switzerland, 1995.